growth of fish has been found much more uniform throughout the year; the changes in the production of plants are there not so marked as in the high Northern latitudes.

Dr. Palmgren and I intend, if circumstances permit, to continue the investigations towards the solution of these problems at the Biological Laboratory of the University of Christiania.

On Blood-Platelets: their Behaviour in "Vitamin A" Deficiency and after "Radiation," and their Relation to Bacterial Infections.

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Introductory. General Effects of Fat-soluble Vitamin Deficiency.

When the fat-soluble vitamin A is withheld from the diet of a rat, the general condition of the animal differs from that resulting from a deficiency of the water-soluble B vitamin. In the latter case the animal ceases to increase in weight almost at once, and then begins to decline. There is a progressive fall in the temperature. The animals always die within a comparatively short time—two months—and are then found to be in a state of profound emaciation, as if they had received no food at all. There is no obvious sign of disease or of an infection. For the sake of convenience, we will designate briefly this condition by the term "marasmus."

The withholding of the fat-soluble vitamin A alone affects different individual rats in a manner as varied and indefinite as the conditions obtained by a deficiency in the water-soluble B are constant and definite. When a young rat is kept on a diet from which the fat-soluble vitamin A is absent, the increase in weight may cease almost at once, or it may continue to increase in weight for many weeks, and almost as rapidly as on a diet containing vitamin A, although eventually its growth will come to a standstill before the full normal size of an adult rat has been reached. We shall, for the sake of convenience, describe these two extremes as the "acute" and the "chronic" effect on growth respectively. Eventually the

rats develop infective conditions, which attack most frequently the eyes, and xerophthalmia develops. There may be other organs affected (septic glands or pneumonia sometimes develop).

These infective conditions develop most rapidly in those rats which show the "acute" effect on growth, when it may first appear after 6–8 weeks. In the rats showing only the "chronic" effect on growth, an infection may not develop at all or very much later. We have kept rats on a fat-soluble vitamin-free diet for four months without any infection appearing, although these rats were kept in the same cage with rats which had developed a very intense xerophthalmia. The external appearance of these rats was, in fact, such as to make them indistinguishable from normal rats. It will be shown, however, that such rats develop the lesion which we consider to be specific for the vitamin A deficiency, although not to the same extent as rats exhibiting the acute effect on growth. The condition of nutrition of rats on a vitamin A-free diet varies, as is to be expected. The rats which show the acute condition are ill-nourished, but the extreme condition of emaciation seen in the rats suffering from the B deficiency is hardly ever met with. Another important difference is that the progressive fall of temperature, which is so characteristic of the B deficiency, does not appear in the A deficiency.

We have been able to control experimentally the conditions under which the "acute" and "chronic" effects of fat-soluble vitamin deficiency appear. Hitherto, the reason for these differences has not been understood. One important factor is the nature of the diet which the rats have received in infancy. If the mother during pregnancy and lactation has received a diet rich in vitamins, and if this diet be given to the young rats after they have been weaned, then the rats are found to be more resistant to a subsequent withdrawal of the fat-soluble vitamin. If, on the other hand, the diet has not been particularly rich in vitamins, although it may contain an amount adequate to maintain the animals in health and to enable them to grow and to breed freely, then withdrawal of the fat-soluble vitamin will produce an immediate and "acute" effect. It may be added that the diet we refer to is not an artificial one, but a natural diet of bread and water, rice and maize, which has for years been used in our laboratory as the standard diet for our stock of rats. Incidentally, this clearly emphasises the great importance of assuring an ample supply of vitamins to the pregnant and lactating woman, and to children, and of not being satisfied with the comfortable belief that our ordinary food contains sufficient vitamins, because we do not suffer from deficiency diseases.

Another factor which determines the onset of xerophthalmia is the amount

of the water-soluble vitamin supplied. When a small amount of this vitamin is given, xerophthalmia develops much earlier than when a large amount is given. This statement refers to experiments in which the water-soluble vitamin was supplied in the form of "marmite."

Between the two extremes which we have described, all intermediate conditions may be observed. These large variations in reaction to a withdrawal of the fat-soluble vitamin make it, of course, very difficult to appraise the significance of any changes in tissues and organs which may be observed. Many changes which can be seen and have been described must be regarded as accidental. For instance, the failure in nutrition which may occur is obviously not an essential feature, and is not necessary for the onset of infective conditions. In connection with our previous work on the relation between lymphoid tissue and nutrition, it is of interest to note that there may be a considerable lymphopenia when the animals have shown the "acute" effect and are in a poor state of nutrition; but when the eye condition develops in a well nourished animal, the lymphoid tissue is normal and the lymphocyte count shows only a slight diminution. The only general feature common to all rats which have been subjected to a fat-soluble vitamin deficiency is a greatly diminished resistance to infection.

We have dealt in considerable detail with the great variety in the general conditions of animals on a vitamin A-free diet, because a lesion, if it is to be considered specific to this deficiency, must be present in all these animals. Further, the severity of such a lesion should be found to vary with the extent to which the animal is affected by this vitamin deficiency. And, lastly, the lesion should disappear when the deficient vitamin is supplied, and the animal recovers as the result.

We have found such a lesion in the great reduction in the number of blood-platelets. We were led to look for a change in the platelets, because we noticed an obvious change in the condition of the blood of rats kept on a diet deficient in the fat-soluble vitamin. When the tail was cut for the purpose of examining the red and white corpuscles, the blood flowed much more freely than in a normal animal, and it was much more difficult to arrest the bleeding. When a film was made, it was more difficult to obtain an even spreading. There were no constant differences in the number of white corpuscles or of the red corpuscles to account for this change, although, as will be seen later, there may be, in advanced stages of the deficiency, a distinct anæmia. But the diminished coagulability of the blood, as it manifests itself by the difficulty of arresting the bleeding, sets in long before the anæmia occurs. The essential importance of the platelets in blood coagulation, which the previous observations of Cramer and Pringle (1)

and of Bordet (2) had demonstrated, led us to examine the blood for platelets.

Method of Counting Platelets in Rats.—The animal is deeply etherised, and the tail placed in a watch-glass filled with a solution of 2 per cent. sodium citrate in 0·6 per cent. NaCl solution. After cutting the tail and allowing the blood to flow until free bleeding is established, the tail is transferred quickly to another watch-glass containing about 1 c.c. of the citrate solution or of Toisson's fluid. Blood is allowed to flow so that a mixture of the solution and blood convenient for counting the red blood corpuscles and the platelets is obtained. After the first few counts it is easy to recognise when the mixture is of convenient concentration. The tail is then wiped dry and the pipette of the hæmocytometer is then filled with blood in the usual way, so as to obtain a count of the absolute number of red corpuscles. In order to count the number of platelets, the citrate-blood mixture is thoroughly stirred, a standard drop placed on a large slide and covered with a cover-glass, which is then cemented with melted paraffin. After allowing the cells to settle, the proportion of red cells to platelets in each field is counted, until about thirty platelets have been counted. From this the absolute number of platelets can be calculated. A helpful device for counting the platelets, which has proved very useful, is as follows: A coarse grating of about 1 mm. squares is ruled on a piece of ground glass with a lead pencil. This is mounted in balsam under a cover-glass, and placed close up to the source of light at right angles to the beam. The image of the grating is then focussed by means of the substage condenser, after the preparation has been focussed with the objective, and conveniently divides the field for counting.

Red Cells and Platelets of normal Rats.—The following Table gives these data for nine normal rats. The counts for the first five rats were made in one laboratory, those of the last four in the other. The figures obtained show

Table I.—Normal Rats.

Weight in grm.	Red cells.	Platelets.
150	10,400,000	960,000
150	11,840,000	730,000
150	10,660,000	845,000
100	10,480,000	720,000
120	9,960,000	660,000
50	8,194,000	786,000
50	10,928,000	1,050,000
60	10,128,000	912,000
60	9,888,000	1,000,000

that the average red cell count for the normal rat lies approximately between 9,000,000 and 10,000,000 cells per cubic millimetre, the average platelet count lies approximately between 700,000 and 900,000 per cubic millimetre. The variations which have been found include not only individual differences of different rats, but also differences due to age, feeding, and those due to the personal factor involved in the technique. The various counts made on rats showing complete recovery from the vitamin A deficiency (see fig. 4 and Table) and from the effects of radium (see fig. 5) give similar figures.

Effect of Vitamin A Deficiency on Platelets.—The rats were kept on a basal ration of casein, starch, autoclaved olive oil and the usual salt-mixture, to which vitamin B was added in the form of marmite. The olive oil prepared as above was known to be free from vitamin A. The casein was freed from the fat-soluble vitamin in some experiments by repeated extraction with alcohol and ether, in others by heating in shallow trays for 24 hours to 130° in air. On such a diet free from the fat-soluble vitamin the platelets show a progressive diminution in their number, and this "thrombopenia"—as it may be called—proceeds *pari passu* with the decline in the general condition of the animal. Thus in the one extreme condition, when the weight of the animal becomes stationary directly the vitamin A is withheld, and when eye symptoms develop within two months, the fall in the number of platelets is rapid and pronounced. Taking the opposite extreme, when the animal continues to grow at an almost normal rate for several weeks, and infective conditions do not make their appearance until much later or not at all, then the fall in the number of platelets is delayed and less pronounced, though still distinct. In fact, a slight fall in the number of platelets may sometimes be the only sign of the vitamin A deficiency, the rat looking quite normal and healthy, and having perhaps only a slightly subnormal weight. Our observations show that infective conditions (xerophthalmia, etc.) do not develop until the platelets have fallen below about 300,000 per cubic millimetre.

It is important to note that the onset of these infective conditions depends on the level to which the platelets have fallen, and not on the length of time to which the rats have suffered from a vitamin A deficiency, nor on exposure to infection, as will be shown presently. When the deficient vitamin A is again supplied after a low platelet count has been established, the number of platelets increases. Here again there is a close parallelism between the rate of increase in the number of platelets and the degree of improvement in the animal.

These statements are based on and illustrated by the experimental data given in the following figures and Tables, which explain themselves and need

little further general comment. In the weight curve of each rat the arrow indicates a count, and the number attached to the arrow gives the number of platelets in thousands. Thus "217" means 217,000 platelets per cubic millimetre. The onset of eye symptoms is indicated by "*x*." More advanced stages are indicated by "*xx*" and "*xxx*." In the figures which illustrate the recovery from the vitamin A deficiency, the broken line represents the weight curve during the last weeks of the absence of the vitamin, the full line gives the weight curve after the addition of this vitamin in the form of cod-liver oil. Most of these recovery curves refer to animals dealt with in the preceding figures, as will be evident from the rat numbers attached to each curve. In these recovery curves the sign (*x*) stands for the complete disappearance of the xerophthalmia.

The figures illustrate all the different varieties of conditions which can be observed in rats when kept on a diet deficient in vitamin A. Special attention is drawn to fig. 1, which refers to an experiment specially devised to illustrate the two extreme conditions and the parallelism between the effect of the vitamin deficiency on the general condition of the animals and on the platelets.

In this experiment two groups of three rats derived from two litters, X and Y, were taken. The litters were born within three days of each other. The mother of the litter X had been kept during pregnancy and lactation on the ordinary laboratory diet of bread and water, rice and maize. This diet was continued for the young rats after they had been weaned until the actual experiment began. The mother of the other litter, Y, had been kept on the same diet, to which an ample supply of vitamins A and B had been added in the form of cod-liver oil and marmite, and this diet also was continued for the young rats.

When the rats were 7 weeks old, the three heaviest and healthy-looking of each litter were selected, and the six rats placed together in one cage and fed with the vitamen-free basal ration (purified casein, starch, olive oil, salt mixture), to which an ample supply of vitamin B was added in the form of crude marmite. As fig. 1 shows, the rats derived from litter X stopped growing almost at once and developed the typical eye infection within 8 or 9 weeks. Those of litter Y continued to grow fairly well at first. After the eleventh week their weight became stationary and remained so for the next 6 weeks (at the time of writing). Up to that time they had not developed any lesion or other infective conditions. They looked perfectly healthy normal rats in a good state of nutrition, and formed a striking contrast with the small, thin, infected rats of litter X. It may be added that the same result was previously obtained in a similar experiment, while in a third such

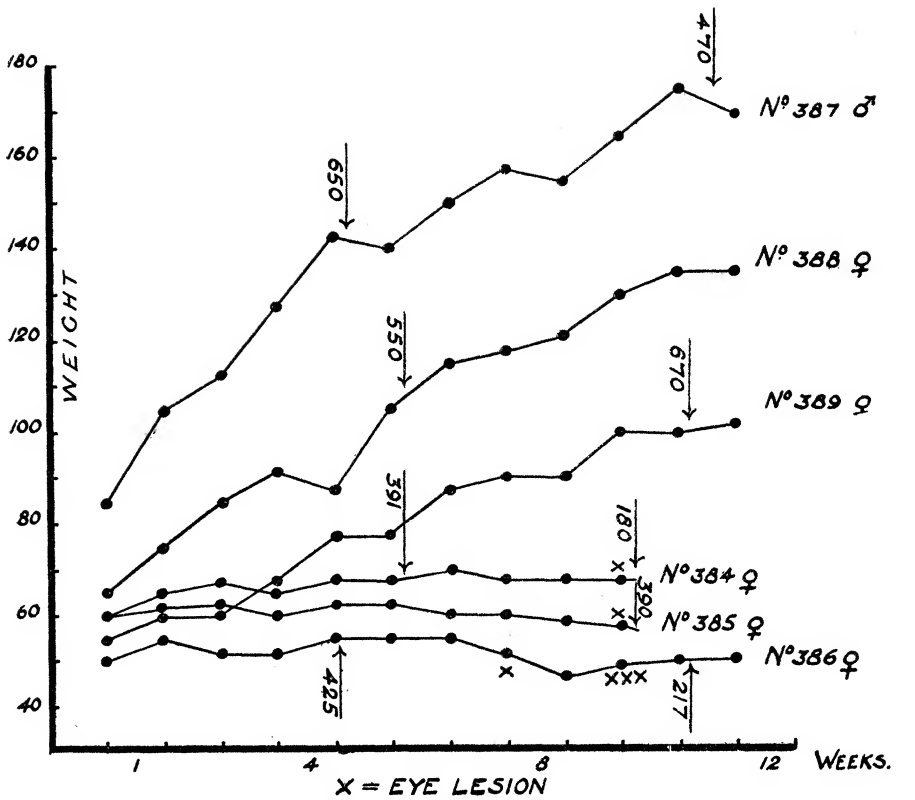

FIG. 1.—Weight curves and platelet count in six rats kept in the same cage on the same vitamin A-free diet. The figure illustrates the parallelism between the degree of thrombopenia and the general condition of the animals.

Table to Fig. 1.

No. of rat.	Weeks of A deficiency.	Red cells.	Platelets.	Condition of rat.
386	4	8,720,000	425,000	No growth.
387	4	7,450,000	650,000	Growing rapidly.
384	5	8,320,000	391,000	No growth.
388	5	8,400,000	550,000	Growing.
384	9	6,200,000	180,000	No growth, thin.
385	9	9,320,000	390,000	Xerophthalmia beginning.
386	10	8,120,000	217,000	Looks very ill. Advanced xerophthalmia.
387	10	9,880,000	470,000	Normal healthy appearance.
389	10	8,800,000	670,000	

experiment the difference, although still present, was not so striking. In the present experiment the blood was examined at different times after the withdrawal of vitamin A in such a way that the number of platelets in a rat of one group could be compared with that of a rat of the other group on the same day. The results, which are arranged in this way in the Table to fig. 1, show clearly that both groups develop a progressive thrombopenia, but that this thrombopenia advances much more rapidly in the severely affected litter X than in litter Y.

Fig. 2 refers to four rats which react in the usual way to the withdrawal of the fat soluble vitamin from the diet, and requires no further explanation.

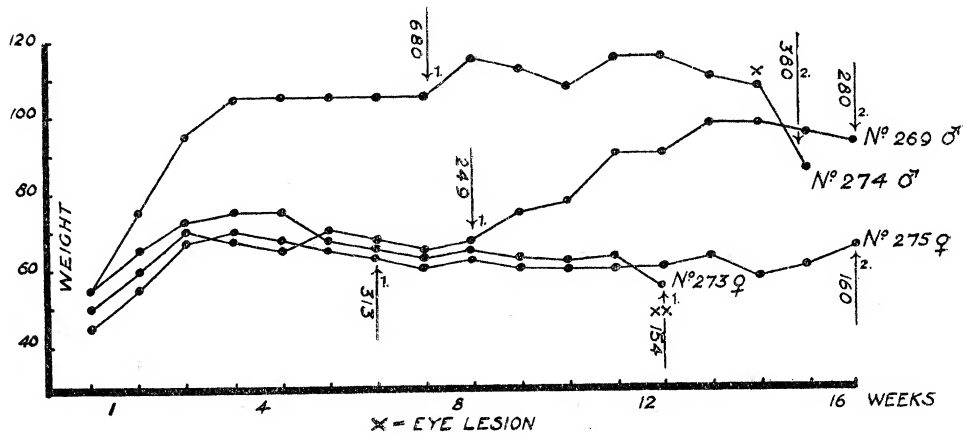

FIG. 2.—Weight curves and platelet count of four rats kept on a vitamin A-free diet.

Table to Fig. 2.

No. of rat.	Weeks of A deficiency.	Red cells.	Platelets.	Condition of rat.
269	8	8,720,000	249,000	
	16	8,240,000	280,000	No eye symptoms.
273	12	6,720,000	154,000	Intense xerophthalmia. Bacteria in blood.
274	7	10,760,000	680,000	
	15	10,320,000	380,000	Xerophthalmia.
275	6	8,000,000	313,000	
	16	7,800,000	160,000	Eye symptoms developed two weeks after this count.

We have stated above that, when A is withheld, the amount of vitamin B supplied determines to a certain extent the onset of the typical symptoms.

Fig. 3 refers to an experiment on three rats in which a minimal amount of vitamin B was supplied. The amount given was sufficient to prevent a fall

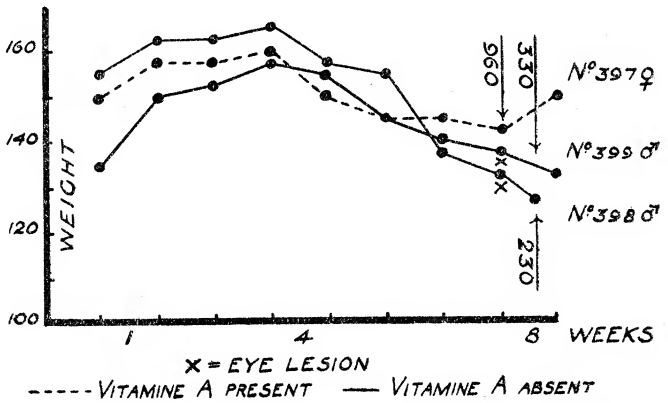

Fig. 3.—Effect of restricting the water-soluble vitamin B to a minimum. Two rats, Nos. 398 and 399, received no fat soluble vitamin, and developed the typical eye lesion. The third rat, No. 397, received an abundant supply of vitamin A, and remained well.

Table to Fig. 3.

No. of Rat.	Weeks of experiment.	Vitamins supplied.	Red cells.	Platelets.	Condition of rat.
398	7	A absent, minimal supply of B	9,680,000	230,000	Xerophthalmia ; died two days later of pneumonia.
399	7		9,400,000	330,000	Xerophthalmia.
397 Control	7	Ample supply of A, minimal supply of B.	10,400,000	960,000	Has not grown, but healthy appearance.

of temperature, but was not sufficient to enable the animals to grow. Two of the three animals, Nos. 398 and 399, received no vitamin A, and they rapidly developed the typical eye lesion, although their weight was over 100 grm. when the experiment began. A third animal, No. 397, received an ample supply of the vitamin A, and served as control. It did not grow, but remained in good health otherwise. In this rat the number of platelets remained normal, while the other two rats developed an intense thrombopenia. Further evidence that the absence of vitamin B does not markedly affect the platelets will be given below.

Perhaps the most striking evidence of the relationship between the fat-

soluble vitamin and the platelets, is afforded by the behaviour of platelets when the animals recover from vitamin A deficiency. This is illustrated in fig. 4. When the fat-soluble vitamin is supplied again, the platelets increase with extraordinary rapidity. Here, again, there is a close parallelism between the recovery from the eye lesion and the increase in the number of platelets. Thus, in rat 277, the eye lesion completely cleared up within a week, and the number of platelets increased from 380,000 to 870,000. In rat 250, with the

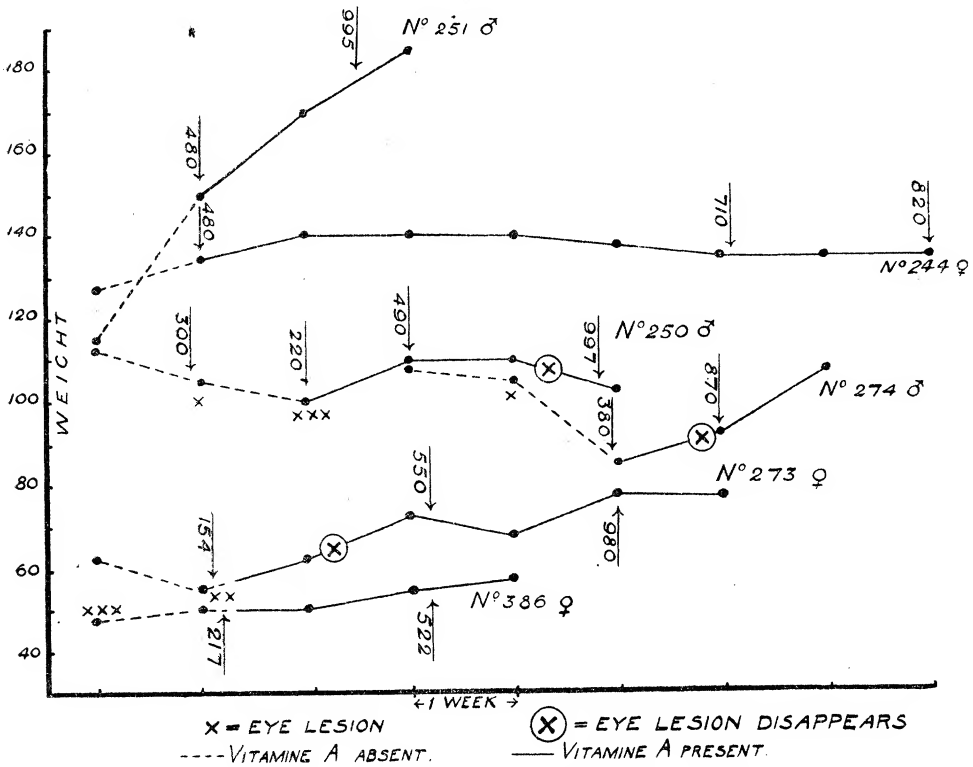

FIG. 4.—Effect of adding vitamin A to the deficient diet. The figure illustrates the parallelism between the rate of increase in the number of platelets and the recovery from infection.

low value of 220,000 platelets and the presence of bacteria in the blood as demonstrated by a film preparation, the platelets increased to almost 1,000,000 within three weeks, the eye lesion became completely cured within two weeks, and the bacteria disappeared from the blood. The reverse condition is represented by rat 386, which was very small and thin and emaciated, and which, in addition to having the eye lesion, developed an abscess in the neck. When cod-liver oil was given the eye lesion cleared up very slowly, and the general nutritive condition of the animal did not improve very much; as the

Table to Fig. 4.

No. of rat.	Deficiency or recovery.	Red cells.	Platelets.	Condition of rat.
244	Deficiency	8,720,000	480,000	
	Recovery, 5 weeks	10,440,000	710,000	
	7 weeks	10,000,000	820,000	
250	Deficiency	9,280,000	300,000	Xerophthalmia, bacteria in blood.
	Deficiency	10,080,000	220,000	Xerophthalmia worse.
	Recovery, 1 week	8,000,000	490,000	Eyes improving.
	3 weeks	9,960,000	997,000	Cured.
251	Deficiency	7,560,000	480,000	
	Recovery, 2 weeks	8,360,000	995,000	
273	Deficiency	6,720,000	154,000	Xerophthalmia.
	Recovery, 2 weeks	9,720,000	550,000	Eyes cured.
	4 weeks	9,760,000	980,000	
274	Deficiency	10,320,000	380,000	Xerophthalmia.
	Recovery, 1 week	10,560,000	870,000	Eyes cured.
386	Deficiency	8,120,000	217,000	Intense xerophthalmia, abscess in neck.
	Recovery, 2 weeks	6,800,000	522,000	Looks thin and ill; xerophthalmia had almost cleared up; abscess still present, but improving.

weight curve also indicates. The platelet count showed only a slow rise from 217,000 to 522,000 after two weeks.

Effect of Vitamin A Deficiency on the Red Cells.—The Tables show that, in the majority of cases, there is no distinct reduction in the number of red cells, even when the platelets are greatly diminished and the animal is in a typical condition of A deficiency. Occasionally, however, an anæmia develops. Rats Nos. 384 and 273 have a distinct anæmia with a red count between 6,000,000 and 7,000,000. These animals also had the most profound thrombopenia. Rat 386 is of interest because it developed an anæmia, not while the vitamin A was withheld, but later on when it was supplied again. It will be recalled that this animal had suffered severely from the deficiency and responded with only an incomplete recovery. The anæmia cannot, therefore, be regarded as the characteristic lesion of vitamin A deficiency. Our observations indicate that these occasional anæmias may follow an infection of the blood with micro-organisms.

Effect of Vitamin A Deficiency on the Leucocytes.—This subject has already been dealt with in a previous paper. There are no constant or characteristic changes. In the final stage, when infection has supervened, there is usually a great increase in the absolute number of polymorphonuclear cells. The lymphocytes show, as a rule, no more than a slight diminution, which contrasts sharply with the profound lymphopenia observed when the water soluble vitamin is withheld. We have already stated that a lymphopenia may also occur in the vitamin A deficiency when the acute effect has been produced and the animals are in a very poor state of nutrition.

There appears to be a change in the number of the polymorphonuclear leucocytes, in the sense that the nucleus is less lobulated in the animals suffering from the vitamin A deficiency. To establish this fact fully would require a very extensive series of observations which we do not propose to undertake. We only refer to it here because it may afford an explanation of the statement that the so-called "Arneth index" (number of lobules of the polymorph nucleus) of tuberculous individuals is higher than that of normal individuals. The explanation may possibly be found in the fact that the high Arneth index of the tuberculous individual is due to his dietary treatment, the diet being very rich in the fat-soluble vitamin.

Effect of Vitamin B Deficiency.—Some observations were made on rats kept on a diet free from vitamin B, but containing an ample supply of vitamin A. The results which are given in the following Table show that when the vitamin B is absent and vitamin A present the platelets do not diminish to any extent, even at a time when the temperature has become very distinctly subnormal, and indicates an advanced stage of the deficiency :—

Effect of B Deficiency.

No. of rat.	Weeks of B deficiency.	Temperature.	Difference of initial weight.	Red cells.	Platelets.
		°			
414	5	35·7	0*g*	11,560,000	980,000
415	5	35·2	0*g*	9,920,000	640,000
416	5	Below 35	−5*g*	10,000,000	1,200,000

In the very last stages of this deficiency we have occasionally obtained low figures for the platelets. But here a technical difficulty arises, because it is difficult in that condition to obtain a free flow of blood when the tail is cut. This is, however, essential, since the blood platelets tend to stick to the tissues of the wound and disintegrate there, when the blood is oozing out slowly.

Even in a normal animal a low count is obtained if, for some reason, a free flow of blood cannot be established.

Effect of Malnutrition Not Due to Vitamin Deficiency.—In order to study this effect young rats of about 50 grm. weight were kept on a protein-free diet, consisting of starch, salt mixture, and olive oil, to which the vitamins A and B were added in the form of cod-liver oil and marmite. As a result the rats decline in weight, but remain otherwise in good health, for three or four weeks. The platelets show no diminution at a time when the animals have lost 10 grm., *i.e.*, 20 per cent. in weight, as the following figures show :—

Effect of Protein Deficiency in the Presence of Vitamins.

No. of rat.	Weeks of protein deficiency.	Temperature.	Loss in weight.	Red cells.	Platelets.
		°			
420	3	38·4	10*g*	9,776,000	1,060,000
421	3	38·3	10*g*	9,280,000	930,000
422	3	38·3	10*g*	10,370,000	1,230,000

Effects of Exposure to Radium.

It is well known that a profound lymphopenia can be produced and maintained by relatively small doses of β or γ radiation. With larger doses additional blood changes occur: first a diminution in the number of polymorphs and with still larger doses a reduction in the number of red cells and hæmoglobin content. For instance, when rats were continuously exposed to radium under constant conditions, a lymphopenia occurred within a few hours, a polymorpho-leucopenia in 7 days and an anæmia in 13 days.

Examples of these effects are given in Protocols Nos. 1 and 2.

Protocol No. 1 showing changes in the polymorphs:—

Three male rats: weights 150, 160, 155 grm., exposed continuously to 220 mgrm. $RaBr_2$, $2H_2O$, distance 8 inches, screen 0·1 mm. lead, 0·12 mm. silver, for 7 days—this is equivalent to 0·55 rads.

Two control rats: weights 210 and 165 grm.

Protocol No. 2 showing changes in red cells and hæmoglobin. Three female rats: weights 80, 85, 75 grm. exposed, continuously to radium, as in Protocol No. 1, for 13 days, equivalent to 1·2 rads.

Three control female rats: weights 80, 85, 75 grm.

The following Table gives the results of the blood examinations:—

Rat.	Weight.	—	Days.						
			0.	2.	7.	11.	16.	23.	30.
Radium	160	P	4·0	2·2	1·5	1·9	1·4	3·0	4·3
		L	10·5	5·4	1·4	1·2	2·4	7·2	7·7
Control	210	P	9·2	7·6	7·4	8·3	5·2	—	—
		L	13·1	15·0	24·0	27·1	20·2	—	—
			0.	2.	4.	8.	14.	18.	25.
Radium	155	P	4·2	4·8	2·2	0·9	1·1	1·0	3·7
		L	20·6	10·2	3·1	1·2	3·4	9·6	10·1
Control	165	P	3·8	3·7	4·6	3·6	5·8	3·2	—
		L	14·7	15·6	23·2	20·2	17·1	20·3	—
			0.	3.	8.	15.	21.	28.	
Radium	150	P	5·3	3·5	1·4	2·6	2·3	3·7	
		L	15·1	4·9	2·0	7·4	9·6	7·4	

P = polymorphs. L = mono-nuclears in thousands per c.mm.

The following Table gives the results of the blood examinations:—

Rat.	Weight.		Days.				
			0.	6.	13.		
Radium	80	R	9·1	8·3	4·1		Dead on 17th day.
		H	102 p.c.	97 p.c.	53 p.c.		
Control	80	R	7·5	8·0	9·1		
		H	98 p.c.	—	105·5 p.c.		
			0.	9.	13.	18.	
Radium	85	R	9·2	7·5	8·0	4·9	Dead on 20th day.
		H	97 p.c.	91·5 p.c.	83 p.c.	69 p.c.	
Control	85	R	8·2	8·5	8·3	10·1	
		H	104·5 p.c.	111 p.c.	101·5 p.c.	115 p.c.	
			0.	13.			
Radium	75	R	7·6	2·1			Dead on 17th day.
		H	96·5 p.c.	—			
Control	75	R	8·5	10·3			
		H	108 p.c.	100 p.c.			

R = red cell content in millions. H = hæmoglobin percentage.

At death these animals exhibited signs of a generalised infection with micro-organisms accompanied by a bronchopneumonia or an enteritis; in one case xerophthalmia was present. If instead of continuing the exposure for 13 days, shorter exposures be given, then an anæmia will either not develop or will supervene after varying lengths of time according to the dose and the weight of the animal; and it is remarkable that so far whenever an animal has developed an anæmia it has invariably died within a few days. This suggests that the anæmia is not directly due to the radiation, but is a secondary effect possibly due to the invasion of the blood stream by micro-organisms. In view of the similarity between these effects and those described above as resulting from withholding vitamin A, an examination of the platelet content of the blood was made to discover whether this could be the primary change to which the invasion of the blood stream by micro-organisms and the anæmia was secondary. The findings are given in the following protocol.

Protocol No. 3.

Five rats: weights 60, 60, 65, 70, 90 grm., exposed as in Protocol No. 1 for 5 days, equivalent to 0·46 rads.

The red cell and hæmoglobin content remained normal in every case.

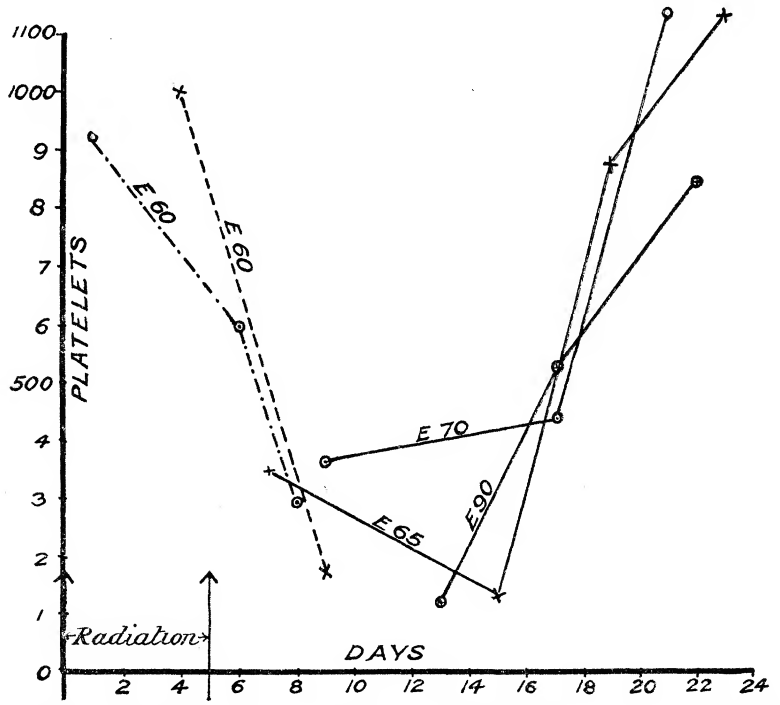

Fig. 5.—Effect of radiation on the number of platelets in five rats. Rapid diminution during and after exposure to radium, followed by rapid recovery.

The changes in the number of the platelets are given in fig. 5, p. 463. A profound thrombopenia develops as a result of the exposure to radium. This persists for a considerable time after the exposure to radium has ceased. It is followed by a rapid spontaneous recovery to the normal number—and sometimes exceeding it—within a week, if the dose of radium has not been too large, as in the present experiment. With larger exposures an anæmia develops and the animals die from intercurrent infections. These findings have an important clinical bearing upon the cases of pernicious anæmia which have occurred among radium workers and which have on a few occasions resulted in death. In all these cases there has been some more or less definite evidence of an infection of the blood stream which has made some hesitate to attribute the condition entirely to exposure to radium. These experiments probably indicate the particular part which micro-organisms play in the manifestation of this type of anæmia; and they indicate the desirability of examining the platelet content in all cases of pernicious anæmia especially of the aplastic varieties.

The Function of Blood Platelets in the Mechanism of Resistance to Bacterial Infection.

The observations recorded in this paper demonstrate a striking relationship between the resistance of an animal to certain bacterial infections and the number of platelets present in the blood. When these latter are diminished below a certain level and kept there for some time, either by withholding the fat soluble vitamin or by exposure to radium, infective conditions develop. When a number of rats are kept in the same cage and on the same vitamin A-free diet, only those animals develop infections in which the platelets have fallen below the critical level. Those rats in which, for reasons given in the paper, the platelets have not been affected to the same extent do not develop these infections. When the deficient vitamin is supplied again the infective conditions clear up as the number of platelets increase, provided of course that these infections have not been allowed to persist for too long a time producing secondary changes such as anæmia, to which the animal eventually succumbs. The infections are as a rule of an avirulent kind. In one case a bacteriological examination was made. Blood cultures from the heart blood showed the presence of two different gram-positive bacilli belonging to the diphtheroid group.

These facts demonstrate that the platelets fulfil an important function in the mechanism of resistance to certain bacterial infections. This conclusion links the observations recorded in this paper to the phenomenon described previously as "defence rupture" or "kataphylaxia" by one of us in conjunc-

tion with Dr. W. E. Gye. Cramer and Gye (3) found that the injection of calcium salts, colloidal silicic acid and other colloids and even distilled water produced at the site of injection a diminution in the resistance to infection. They showed subsequently (4) that all these different substances have in common that they produce the same lesion: a damage to the endothelium of the smaller blood-vessels which elicits an agglutination of the platelets within the vessel, and the formation of a white thrombus with a resulting local disturbance in the circulation (see Plate VI, fig. 3, in VI. Scientific Report of Imperial Cancer Research Fund). At the same time lymph and plasma pass out into the surrounding connective tissue where they form a gelatinous clot. When the washed bacteria of gas gangrene or tetanus are injected at the site of this lesion the specific disease (gas gangrene or tetanus as the case may be) is elicited in a very virulent form at this site. The same bacteria when injected into a different site of the same animal do not elicit the disease there but, as in a normal animal, undergo phagocytosis and lysis. At the site of the lesion active phagocytosis is still proceeding, but evidence of lysis has never been observed. This phenomenon of defence rupture is not restricted to the anaërobic bacteria of gas gangrene and tetanus, but holds good also for streptococci, and has recently been shown by Gye and Kettle to be valid also for tubercle bacilli. The lesion responsible for this phenomenon is one which puts the platelets "out of commission," so to speak, *locally* by agglutinating them, and which, by its interference with the circulation, prevents the access of new platelets. This leads to a *local* diminution in the resistance to infection. In the thrombopenia of vitamin A deficiency, or after exposure to radium, there is a *general* absence of platelets, and this leads to a *general* diminution in the resistance to infection.

The literature contains some statements which afford direct evidence that the platelets are concerned in the elaboration of bactericidal substances. The washed platelets and leucocytes do not contain any bactericidal substances when tested against the anthrax bacillus. But when they are mixed with tissue fluids which, by themselves, are inactive, they confer upon this fluid an intense bactericidal power (Gruber and Futaki (5)). It should be noted that these bactericidal substances are not identical with the hæmolytic complement.

More recent work would appear to assign to the platelets a somewhat different function. C. G. Bull (6) has shown in a series of papers that certain bacteria (staphylococci, colon bacilli, meningococci, typhoid bacilli, non-virulent pneumococci and non-virulent influenza bacilli) are rapidly agglutinated when injected into the blood of a normal rabbit or dog. This is followed by a rapid removal of the bacteria from the circulation and by phagocytosis and destruction of the agglutinated bacteria in the

capillary systems of the viscera. Those bacteria which are not agglutinated remain in the circulation and produce a progressive septicæmia. Generally speaking, comparing different types of bacteria, the degree of the agglutination of the infecting bacteria in the circulation of the host is a measure of the resistance of the host to the particular types of organism. Bull drew special attention to the fact that with typhoid bacilli, for instance, the mechanism of defence in the living body is very different from that observed *in vitro* by serum or defibrinated blood. In the latter destruction is caused by bacteriolysis, while in the living animal there is the process of agglutination and subsequent phagocytosis in the organs described above. Delrez and Govaerts (7, 8) have followed up these observations, and have shown that this process of agglutination in the living animal is brought about by the platelets. They found that a few minutes after the injection of certain bacteria there is an agglomeration of the platelets and the bacteria. A few minutes later the masses of agglomerated bacteria and platelets can be found in the liver undergoing phagocytosis.

The thrombopenia, which is produced in guinea-pigs by the injection of an antiplatelet serum (the experimental purpura of Ledingham (9)), does not lead to the development of infective conditions, because the animals either die within a few days as the result of the hæmorrhages, or when they recover the thrombopenia rapidly passes off. The thrombopenia alone is, as Bedson (10) has shown, not sufficient to produce hæmorrhages. These are the combined result of the thrombopenia and of a lesion of the vascular endothelium produced by the antiplatelet serum.

Summary.

The absence of the fat soluble vitamin from the diet always leads in the rat to a progressive diminution in the number of blood platelets. This thrombopenia is the only constant lesion which we have found, so far, in every case of vitamin A deficiency, and we regard it as the lesion characteristic of this deficiency, just as the lymphopenia is characteristic of the vitamin B deficiency. A thrombopenia may even be found in rats kept on a vitamin A free diet when they do not yet show any obvious signs of ill-health, and are, to all external appearances, normal animals. When a profound thrombopenia has been established, the addition of the missing vitamin A to the diet is followed by a rapid increase in the number of platelets to the normal number, provided that the animal has not been allowed to suffer too long and too severely from the deficiency.

Exposure to radium produces not only a lymphopenia, but also with sufficiently large doses a thrombopenia. From this the animals recover

rapidly, if the application of radium is discontinued, and the dose has not been too large.

If by exposure to radium, or by withholding the fat soluble vitamin, the number of platelets has been reduced below a certain critical level—about 300,000 for the rat—the resistance of the animal to infection is greatly diminished and infective conditions develop spontaneously. These may lead to a secondary anæmia. The infective conditions may clear up again as the number of platelets is made to increase.

The blood platelets fulfil an important function in the mechanism of resistance to bacterial infection. Alterations in the local or general resistance to infection are associated with local or general changes in the distribution of the platelets.

The radium used in these investigations was a loan from the Medical Research Council.

REFERENCES.

(1) Cramer and Pringle, 'Phys. Soc. Proc.,' July 27, 1912, p. 11 ; 'Journ. of Phys.,' vol. 45 ; 'Quart. Journ. Exp. Physiology,' vol. 6, p. 1 (1913).
(2) Bordet et Delange, 'Annales de l'Institut Pasteur,' vol. 26, p. 657 (1912).
(3) Bullock and Cramer, 'Roy. Soc. Proc.,' B, vol. 90, p. 513 (1919).
(4) Bullock and Cramer, 'VI. Scientific Report Imperial Cancer Research Fund.' Taylor and Francis, 1919.
(5) Gruber and Futaki, 'München. Med. Wochenschr.,' 1907, p. 249 ; 'Deutsche Med. Wochenschr.,' 1907, p. 1588.
(6) Bull, 'Journ. Exp. Med.,' vol. 20, p. 236 (1914) ; vol. 22, pp. 475 and 484 (1915) ; vol. 23, p. 419 (1916) ; vol. 24, p. 25 (1916).
(7) Delrez et Govaerts, 'Compt. Rend. Soc. de Biol.,' vol. 81, p. 53 (1918).
(8) Govaerts, *ibid.*, vol. 85, p. 745 (1921).
(9) Ledingham, 'The Lancet,' 1914, p. 1673 ; vol. 1, p. 311 (1915).
(10) Bedson, 'Journ. Path. and Bact.,' vol. 25, p. 94 (1922).